California Institute for Regenerative Medicine

Alternative splicing events identified in human embryonic stem cells and neural progenitors.

Public Summary:

Scientific Abstract:

Human embryonic stem cells (hESCs) and neural progenitor (NP) cells are excellent models for recapitulating early neuronal development in vitro, and are key to establishing strategies for the treatment of degenerative disorders. While much effort had been undertaken to analyze transcriptional and epigenetic differences during the transition of hESC to NP, very little work has been performed to understand post-transcriptional changes during neuronal differentiation. Alternative RNA splicing (AS), a major form of post-transcriptional gene regulation, is important in mammalian development and neuronal function. Human ESC, hESC-derived NP, and human central nervous system stem cells were compared using Affymetrix exon arrays. We introduced an outlier detection approach, REAP (Regression-based Exon Array Protocol), to identify 1,737 internal exons that are predicted to undergo AS in NP compared to hESC. Experimental validation of REAP-predicted AS events indicated a threshold-dependent sensitivity ranging from 56% to 69%, at a specificity of 77% to 96%. REAP predictions significantly overlapped sets of alternative events identified using expressed sequence tags and evolutionarily conserved AS events. Our results also reveal that focusing on differentially expressed genes between hESC and NP will overlook 14% of potential AS genes. In addition, we found that REAP predictions are enriched in genes encoding serine/threonine kinase and helicase activities. An example is a REAP-predicted alternative exon in the SLK (serine/threonine kinase 2) gene that is differentially included in hESC, but skipped in NP as well as in other differentiated tissues. Lastly, comparative sequence analysis revealed conserved intronic cis-regulatory elements such as the FOX1/2 binding site GCAUG as being proximal to candidate AS exons, suggesting that FOX1/2 may participate in the regulation of AS in NP and hESC. In summary, a new methodology for exon array analysis was introduced, leading to new insights into the complexity of AS in human embryonic stem cells and their transition to neural stem cells.